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Testing the Critical Window Hypothesis in Alzheimer's Disease: brain vs gut mechanisms of estrogen therapy timing

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Abstract
The decline in estrogen after menopause is linked to increased risk of cognitive decline and Alzheimer's Disease (AD) in women. Estrogen supports neuronal metabolism, synaptic plasticity, and anti-inflammatory signaling in the brain while also regulating gut microbiome composition, suggesting that its loss may influence AD risk through both brain-centered mechanisms and gut–brain axis pathways. This review examines current evidence on estrogen-based Hormone Replacement Therapy (HRT) and its effects on cognitive function, Alzheimer's risk, and gut microbial diversity in postmenopausal women, with emphasis on the critical window hypothesis. Existing research indicates that oral estrogen-only therapy initiated within ~five years of menopause is most consistently associated with reduced AD risk, whereas later initiation or combined estrogen–progesterone therapies show weaker or inconsistent benefits. Emerging studies also suggest estrogen therapy may help preserve gut microbial diversity and limit dysbiosis, a microbial imbalance frequently observed in Alzheimer's disease and linked to neuroinflammatory pathways. Despite these findings, the biological basis of the critical window remains unresolved—specifically whether the loss of hormone therapy benefit over time is driven primarily by neuronal estrogen receptor changes in the brain or by alterations in the gut microbiome. Determining whether the critical window arises from gut microbiome dysbiosis or brain-specific loss of estrogen responsiveness would have immediate therapeutic implications: microbiome-driven mechanisms could potentially be reversed through probiotic or microbiome-targeted interventions, whereas brain-driven mechanisms would require therapies that bypass diminished neuronal estrogen signaling. This paper proposes a timing-stratified randomized estrogen intervention study measuring brain biomarkers of neurodegeneration and gut microbiome responses across early, mid, and late postmenopausal stages while controlling for genetic risk factors. By comparing brain and gut responses to estrogen therapy, the proposed study aims to determine whether the critical window arises from brain-specific loss of estrogen responsiveness or gut-mediated mechanisms, guiding more precise strategies to reduce Alzheimer's disease risk in postmenopausal women.

Keywords
Estrogen, Hormone Replacement Therapy (HRT), Critical Window Hypothesis, Alzheimer's Disease, Menopause, Gut microbiome, Gut–brain axis, Neuroinflammation, Microbiome dysbiosis, Postmenopausal women

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1. Introduction

1.1 Background

Menopause is a natural, biological process that signifies the end of a woman's reproductive years. This transition is marked by a significant decline in estrogen levels. Estrogen is a hormone that plays a vital role in not only the reproductive functioning of the body, but also in the regulation of brain and gut health. As estrogen levels drop, and as brain and gut health subsequently decline, postmenopausal women become increasingly vulnerable to heightened risks for cognitive decline and neurodegenerative diseases such as Alzheimer's Disease (AD) (1). In fact, women comprise two-thirds of Americans living with AD—a disparity that highlights the sex-specific risk of AD and has prompted researchers to look beyond genetics or aging as the only risk factors, instead examining hormonal changes that occur in women's bodies after menopause (1).

Alzheimer's is a neurodegenerative condition that is thought to occur when the brain's neurons undergo degeneration and begin to die, leading to progressive cognitive decline. A healthy adult brain has billions of neurons, each with long, branching extensions (2). These extensions allow for individual neurons to form connections with other neurons. These connections, called synapses, are the points where signals flow from one neuron to another. At each synapse, a neuron releases signals in the form of tiny bursts of chemicals — neurotransmitters — which are then received by neighboring neurons. The brain contains trillions of synapses, which allow signals to travel rapidly throughout the brain. These signals encode and convey information underlying cognitive and physiological functions, including memory, thought, sensation, emotion, movement, and learned skills (2). Within the amyloid-beta hypothesis of AD, two major pathological changes disrupt synaptic communication and contribute to the neuronal damage and cell death characteristic of the disease: the accumulation of beta-amyloid protein fragments into extracellular aggregates known as beta-amyloid plaques, and the buildup of an abnormal form of the protein tau inside neurons, forming neurofibrillary tangles (2). Another key pathological change believed to contribute to the progression of AD is inflammation, particularly neuroinflammation (2).

Hormone therapy has emerged as a potential intervention to restore estrogen levels in women and may help reduce the risk of AD. A growing body of research suggests that, when administered under appropriate conditions, hormone therapy may lower AD risk through two primary mechanisms: improving cognitive function and restoring balance in the gut microbiome (3, 4). Although the decline in estrogen has long been known to affect cognitive health, its potential impact on the gut —and its role in reducing AD risk—has only recently emerged as an area of active research. Only in recent years have studies begun to investigate the effects of hormone therapy on the gut. Estrogen's ability to influence the composition and diversity of the gut microbiome is important because it may help prevent gut dysbiosis. Dysbiosis can disrupt neural pathways such as the gut–brain axis, and such disruptions have been associated with an increased risk of neurodegenerative diseases (5, 6). While previous studies have examined hormone therapy's effects on cognitive decline and gut microbiome composition separately,

few analyses have considered how these systems interact in determining Alzheimer's disease risk in postmenopausal women. This review synthesizes current evidence across these domains and proposes an experimentally testable framework to determine whether the critical window for estrogen therapy arises primarily from brain-specific mechanisms or gut microbiome–mediated pathways.

1.2 Methods

This literature review was conducted to examine the effects of hormone therapy on cognitive health and gut microbiome diversity, particularly focusing on reducing AD risk, in postmenopausal women. The literature search was conducted within the PubMed database using combinations of keywords, including "menopause", "postmenopause", "estrogen", "hormone therapy", "estrogen therapy", "Alzheimer's disease", "cognitive decline", "gut microbiome", "gut dysbiosis", and "gut–brain axis".The initial search returned ~ 35 articles across the keyword combinations. Titles and abstracts were screened for relevance before full-text review, and the most relevant studies were selected for inclusion in the paper.

Inclusion criteria included peer-reviewed studies published within the past 10–15 years examining menopause, estrogen decline, hormone therapy, Alzheimer's disease risk, cognitive outcomes, or gut microbiome changes. Priority was given to human studies involving postmenopausal women, as well as systematic reviews, meta-analyses, and observational or randomized clinical studies. Relevant animal studies were included when necessary to explain biological mechanisms.

Exclusion criteria included non-peer-reviewed sources, duplicate studies, and articles not directly related to menopause, Alzheimer's disease, or gut microbiome interactions.

2. Analysis of prior work

2.1 Estrogen's role in brain health and link to AD

Estrogen is essential in maintaining cognitive health by modulating multiple mechanisms: glucose metabolism, neuronal plasticity, oxidative stress, and neural inflammation (1). Glucose metabolism occurs when carbohydrates and proteins are broken down into glucose, a sugar that serves as the body's primary metabolic fuel (7). Through estrogen receptors —particularly abundant in the hippocampus and prefrontal cortex, regions critical for memory and cognitive processing—estrogen enhances glucose transport and utilization in the brain, thereby helping maintain adequate neuronal energy levels (1). Neuronal plasticity refers to the nervous system's ability to modify itself in response to experience and injury in terms of its function and structure; it underlies an individual's ability to remember and learn from past experiences (8). Neuronal plasticity can be strengthened through synaptogenesis—the formation of new synaptic connections between neurons—a process that estrogen has been shown to promote (1). Consequently, estrogen plays a critical role in supporting learning and memory (1). Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS), also known as free radicals, and the body's antioxidant defenses (9). When ROS production exceeds the capacity of antioxidant systems, these molecules accumulate and can damage cellular components such as DNA, lipids, and proteins. This damage is particularly harmful in the brain

and is considered a major contributor to neurodegeneration (1, 9). Oxidative balance is particularly critical in the brain, and estrogen helps regulate mitochondrial function to enhance the brain's antioxidant defenses, thereby reducing ROS levels and the risk of oxidative damage (1). Lastly, neuroinflammation occurs when immune responses trigger inflammation in the brain and spinal cord (10). Estrogen helps reduce neuroinflammation—a key contributor to neurodegeneration—partly by promoting neurogenesis, the formation of new neurons, which may help replace neurons damaged or impaired by inflammatory processes (1). The decline of the cognitive and neuroprotective processes described above—processes that are supported by estrogen—represents an important risk factor for the development of AD. Consequently, the loss of estrogen may increase the risk of AD pathogenesis through overlapping mechanisms that link estrogen decline with neurodegenerative processes. To better understand estrogen's role in AD progression, researchers often focus on the physiological changes that occur during the menopausal transition.

Women are at risk of developing dementia and Alzheimer's due to perimenopause and menopause, as supported by the fact that women comprise two-thirds of all Alzheimer's cases (1). Estrogen loss most often occurs during the time of menopause in the female body. Menopause is defined as the point in time when a woman has not had a menstrual period for 12 consecutive months (1). In the United States, the average age of menopause is ~ 51 years; however, the age of menopause often naturally occurs anywhere between the ages of 45 and 55 (1). As previously mentioned, during this time, estrogen and progesterone levels drop in a woman's body, resulting in the termination of ovulation and, therefore, of menstruation (1). The menopausal transition, known as perimenopause, is the phase preceding menopause during which a woman's body undergoes significant hormonal changes (1). This period can last from several months to several years and typically begins in a woman's late 30s or 40s (1). Because AD develops later in life, its early pathological processes may overlap with the hormonal changes that occur during perimenopause and menopause. As these stages involve a substantial decline in estrogen levels, they may represent an important period of increased vulnerability to AD. The risk of dementia—one of the most common clinical manifestations of Alzheimer's—also increases during this time.

2.2 Hormone Replacement Therapy

Hormone therapy, specifically Hormone Replacement Therapy (HRT), is a potential protective intervention that can help to replace hormones such as estrogen, which are lacking in the body. There are two main types of HRT; estrogen therapy and combination estrogen and progesterone therapy. The potential benefits of HRT arise largely from the ability of estrogen to restore its neuroprotective effects (1). There is evidence that certain estrogen formulas used in HRT, specifically those containing 17β-estradiol (E2), are associated with a reduced risk of AD (1). When administered to otherwise healthy postmenopausal women aged 50–62, HRT reduced cognitive decline, particularly the decline in verbal memory. However, no comparable benefit was observed in older women. These findings suggest that the effects of HRT may depend on factors such as the age at initiation and the time elapsed since

menopause or perimenopause, supporting the concept that the timing of hormone therapy influences its cognitive outcomes (1).

2.2.1 *Risks and benefits of HRT*

Research determining whether or not HRT is successful in decreasing the risk of dementia and Alzheimer's in women has provided mixed results. Song et al. performed a systematic review and meta-analysis of 3 randomized controlled trials, 12 cohort studies, and 16 case-control studies involving ~7 million women. They reported an association between menopausal hormone therapy (MHT) and a reduced risk of AD (4). However, this relationship appeared to depend strongly on the timing of therapy initiation. When MHT was initiated within approximately 3–5 years of menopause, a lower risk of AD was observed (4). In contrast, the use of combined estrogen–progesterone MHT or progesterone-only MHT was associated with an increased risk of AD. In these cases, the reported odds ratio (OR) was 1.13, indicating a 13% higher likelihood of developing AD compared with women who did not use these therapies (4). Differences were also observed across administration methods: oral estrogen-only MHT was associated with a greater reduction in AD risk than alternative delivery methods such as transdermal therapy (4). Together, these findings support the "critical window" or "timing hypothesis," which proposes that the neurological effects of hormone therapy depend on how soon treatment is initiated relative to menopause.

A systematic review and meta-analysis by Nerattini et al. (2023), which included six randomized controlled trials (RCTs) involving 21,065 treated participants and 20,997 placebo participants, as well as 45 observational studies comprising 768,866 patient cases and ~ 5.5 million controls, reported important findings regarding the relationship between HRT and the risk of AD (11). The observational studies suggested a reduced risk of AD and all-cause dementia among women using hormone therapy, with particularly protective associations observed for estrogen-only therapy (11). The review also identified timing as an important factor influencing dementia risk. Specifically, midlife use of estrogen-only hormone therapy was associated with an estimated 32% reduction in the risk of dementia. However, the authors note that this estimate should be interpreted cautiously due to methodological limitations in the underlying analyses. Nevertheless, the findings provide a meaningful estimate of potential protective effects. In contrast, among postmenopausal women aged 65 years and older, the use of combined estrogen–progesterone therapy was associated with a significantly increased risk of developing dementia (11). No significant change in dementia risk was observed when estrogen-only hormone therapy was used in this older population (11).

The literature hence suggests that estrogen-only hormone therapy—particularly when administered orally and initiated within five years of menopause—is associated with a lower observed risk of developing both AD and dementia. In contrast, combined estrogen–progesterone therapy or progesterone-only therapy has been associated with an increased risk of AD, particularly when initiated later in life, such as in women aged 65 years or older. Both reviews emphasize the importance of personalizing MHT, also referred to as HRT, based on individual risk factors and treatment timing. The findings regarding the importance

of therapy timing further support the critical window hypothesis, which proposes that the neurological effects of hormone therapy depend on when treatment is initiated relative to age and the menopausal transition, with the most beneficial outcomes observed when therapy begins earlier; i.e. within 5 years of menopause (12).

2.3 Menopause-related changes in gut microbial diversity and the resulting dysbiosis

The decline in circulating estrogen following menopause also has important effects on other physiological systems, particularly the gut microbiome. The gut microbiome refers to the trillions of microorganisms—primarily bacteria—that inhabit the human gastrointestinal tract. These microbial communities are involved in a wide range of biological processes, including metabolic regulation, immune function, mood regulation, and, most notably, brain function and cognition through the gut–brain axis (5). Microbes in the gut break down complex dietary carbohydrates, producing Short-Chain Fatty Acids (SCFAs) as metabolic byproducts (5). These SCFAs are absorbed by epithelial cells lining the intestinal mucosa—the innermost layer of the gastrointestinal tract—and subsequently distributed throughout the body to organs such as the liver, brain, and heart, where they influence a variety of physiological processes (5).

Declining levels of estrogen and progesterone following menopause can lead to physiological changes in the gut, including reduced microbial diversity (5). Communication between the gut microbiome and the brain is thought to occur in part through the vagus nerve, a major component of the parasympathetic nervous system. Many gut microbes produce secondary metabolites that can interact with the nervous system, allowing signals to travel between the gut and the brain (5). This bidirectional communication pathway is known as the gut–brain axis. As a result, the composition of the gut microbiome has become an increasingly important area of research and is now being investigated as a potential biomarker of overall health and disease risk (5).

Estrogen interacts with the gut microbiome through a complex, bidirectional regulatory relationship (5). After being synthesized in the ovaries, estrogens circulate throughout the body and act on a variety of tissues (5). They are subsequently metabolized in the liver and excreted into bile—a digestive fluid stored in the gallbladder—and released into the intestine (5). In the gut, microbial enzymes such as β-glucuronidase can deconjugate estrogen metabolites, converting them back into active forms that may be reabsorbed into the bloodstream through enterohepatic circulation (5). Through this process, the gut microbiome directly influences the amount of circulating estrogen in the body. This microbial recycling of estrogen is thought to contribute to maintaining intestinal tissue integrity and supporting estrogenic signaling throughout the body (5).

In postmenopausal women, enterohepatic circulation becomes an important mechanism for maintaining circulating estrogen levels, as ovarian estrogen production declines (5). However, although this recycling pathway can help preserve some estrogen activity, it cannot fully compensate for the substantial reduction in ovarian estrogen following menopause. Reduced estrogen levels may therefore contribute to intestinal inflammation and

disruptions in microbial balance, which can impair gut barrier function and alter communication along the gut–brain axis (5).

The shift in the gut microbiome during menopause, together with the decline in estrogen activity, can have disruptive physiological effects. For example, the gut microbiome of postmenopausal women may begin to resemble that of age-matched men, as microbial diversity often declines following menopause (5). Reduced microbial diversity, combined with inflammation and compromised intestinal tissue integrity, can contribute to increased gut permeability. This effect may be further exacerbated by the continued decline in estrogen levels, which can impair the body's ability to repair and maintain intestinal barrier function (13). The term dysbiosis refers to an imbalance in microbial communities (13). In the gut, dysbiosis occurs when the composition of microbial populations is disrupted in ways that impair functions essential for maintaining health or promote processes that contribute to disease. Such disruptions can arise when microbial diversity is reduced or when harmful microbial species become dominant (13).

2.4 Gut microbiome dysbiosis in Alzheimer's and cognitive decline

The discussion of gut microbiome dysbiosis following menopause and declining estrogen levels raises the question of whether gut microbial imbalance may also serve as a marker of cognitive decline and AD. A systematic review and meta-analysis by Jemimah et al. (2023), which analyzed 17 studies including 679 patients with AD or mild cognitive impairment (MCI)—a condition often considered a precursor to AD—found that perturbations in gut microbiome composition, referred to as dysbiosis, were associated with several neurodegenerative disorders (6).

The proposed pathway linking gut dysbiosis to neurodegeneration involves a sequence of physiological processes, including immune activation through a compromised intestinal barrier, the induction of systemic inflammation, impairment of the blood–brain barrier, and subsequent neuroinflammation (6). Immune activation through a compromised gut barrier occurs when the intestinal lining becomes weakened or "leaky," allowing toxins, bacteria, or other harmful substances to enter the bloodstream and trigger immune responses (14). Once activated, the immune system can initiate a systemic inflammatory response, resulting in inflammation throughout the body. Through signaling along the gut–brain axis, these inflammatory signals may influence brain function.

Systemic inflammation can also impair the blood–brain barrier, which normally protects the brain by preventing harmful substances circulating in the blood from entering neural tissue (14). When this barrier is compromised, inflammatory molecules and toxins may reach the brain more easily, contributing to neuroinflammation (14). Chronic neuroinflammation is considered a key factor in the development of neurodegenerative diseases, including AD, as it can promote the accumulation of amyloid-β plaques, tau pathology, and neuronal damage (15).

Consistent with this mechanism, reduced microbial diversity and altered gut microbial composition are frequently observed in individuals with AD. Such changes in microbial communities—characteristic of gut dysbiosis—

are increasingly being investigated as potential biomarkers of neurodegenerative disease progression.

Meta-analyses of the literature (mean age ≈71 years; 61.9% women) have reported reduced bacterial species richness and diversity in the gut microbiome of individuals with AD (6). These studies also sought to identify specific bacterial taxa that differed in abundance between AD patients and control groups and to evaluate their associations with disease risk (6).

Notably, reduced abundances of members of the Lachnospiraceae family (including the genera *Lachnospira* and *Anaerostipes*) and the Bacteroidaceae family (genus *Bacteroides*) were observed in the gut microbiomes of AD patients (6). Bacteria belonging to the Lachnospiraceae family are known to break down complex polysaccharides through hydrolytic processes, producing short-chain fatty acids (SCFAs) such as butyrate (16). These microbial metabolites play important roles in maintaining intestinal barrier integrity and regulating several physiological pathways, including immune and neural signaling.

Members of the *Bacteroides* genus are among the dominant beneficial bacteria in the human gut microbiome (17). In the gastrointestinal tract, they contribute to host health by helping protect against pathogenic microbes and by supplying nutrients that support other microbial communities. However, notable regional differences were observed in the abundance of *Bacteroides* across study populations (6). Specifically, meta-analyses reported reduced *Bacteroides* abundance in Chinese cohorts but increased levels in U.S.-based AD cohorts (6). These findings suggest that factors such as geographic region, diet, and lifestyle may significantly influence gut microbial composition and may therefore contribute to variability in microbiome associations with AD (6).

Furthermore, researchers observed an increased abundance of the Bifidobacteriaceae family, particularly the genus *Bifidobacterium*, in individuals with AD (6). Members of the *Bifidobacterium* genus typically metabolize carbohydrates such as galactose and play important roles in maintaining intestinal and immune health (16). Reduced levels of these bacteria have been associated with systemic and intestinal inflammation, both of which are linked to chronic inflammatory conditions that are considered risk factors for neurodegenerative diseases, including AD (16). Although the observed increase in *Bifidobacterium* might initially appear beneficial, it may represent a compensatory response to the loss of other beneficial microbial species. Such changes may therefore reflect the broader microbial imbalance characteristic of gut dysbiosis in AD patients.

Other bacterial families reported to be increased in abundance in the gut microbiomes of AD patients include Enterobacteriaceae and Lactobacillaceae (6). Members of the Enterobacteriaceae family are Gram-negative, non–spore-forming bacteria that are widely distributed in environments such as soil, water, and the gastrointestinal tract (18). While some species exist as normal components of the gut microbiota, others are associated with intestinal and extraintestinal infections in humans (18). An increased abundance of Enterobacteriaceae in the gut microbiome may therefore reflect microbial imbalance or compromised intestinal

health.

Bacteria belonging to the Lactobacillaceae family, a group of lactic acid–producing microbes, also play diverse roles within the gut microbiota (19). Many species within this family contribute to intestinal health by modulating immune responses, maintaining microbial balance, and protecting against pathogenic organisms, which is why they are commonly used as probiotic organisms (19). However, similar to the previously observed increase in *Bifidobacterium* from the Bifidobacteriaceae family, elevated levels of Lactobacillaceae in AD patients may represent a compensatory response to broader disruptions in gut microbial composition. Such shifts further highlight the substantial alterations in gut microbiota observed in individuals with neurodegenerative diseases such as AD.

Moreover, increased abundances of bacterial genera such as *Clostridium* and *Akkermansia* have also been reported in the gut microbiomes of individuals with AD (6). The genus *Clostridium* includes a diverse group of bacteria with varying effects on human health. While some species may have neutral or beneficial roles in the gut ecosystem, others are associated with disease. For example, certain pathogenic strains, such as *Clostridioides difficile*, can cause severe gastrointestinal infections (20). Consequently, an increased abundance of bacteria within this group may indicate microbial imbalance and a greater potential for harmful strains to proliferate. Bacteria belonging to the genus *Akkermansia* have been shown in some studies to play potentially beneficial roles in intestinal health, including modulating metabolic and inflammatory processes (21). However, similar to the increases observed in members of the Bifidobacteriaceae and Lactobacillaceae families, elevated levels of *Akkermansia* may also represent a compensatory response to the loss of other beneficial microbial species. Such shifts further highlight the substantial alterations in gut microbial composition associated with AD.

In conclusion, the reduced microbial diversity observed in the gut microbiomes of AD patients, together with increased abundances of potentially harmful or pro-inflammatory bacterial taxa, supports the hypothesis that gut dysbiosis may contribute to the development and progression of AD. In particular, reductions in the Lachnospiraceae and Bacteroidaceae families—both of which include species known to produce beneficial SCFAs and help protect the host from pathogenic microbes—suggest a weakening of the gut's protective and metabolic functions. Alongside the loss of these beneficial microbial groups, increased abundances of taxa such as Enterobacteriaceae, Lactobacillaceae, and genera including *Clostridium* and *Akkermansia* have also been reported in individuals with AD. These changes may reflect either the proliferation of potentially harmful bacteria or compensatory shifts within the microbial ecosystem in response to the loss of beneficial species. Together, these microbial alterations highlight the significant disruption of gut microbial balance associated with AD.

2.5 How hormone therapy may restore gut health

Hormone replacement therapy (HRT) has been associated with increased circulating estrogen levels in postmenopausal women and may therefore contribute to a reduced risk of AD. Restoring estrogen levels may also help

maintain gut microbial balance by promoting microbial diversity and reducing the likelihood of gut dysbiosis. As a result, hormone therapy may represent a potential strategy for improving gut health in postmenopausal women.

To investigate this relationship, Leite et al. (2022) analyzed data from 35 female participants enrolled in the REIMAGINE study, a large-scale research project conducted at Cedars-Sinai Medical Center. REIMAGINE—*Revealing the Entire Intestinal Microbiota and its Associations with the Genetic, Immunologic, and Neuroendocrine Ecosystem*—aims to characterize the small-intestinal (duodenal) microbiome and its associations with various health conditions (3).

Participants were divided into three groups: postmenopausal women receiving hormone therapy (HT+), postmenopausal women not receiving hormone therapy (HT−), and women of reproductive age (RA). The HT+ group included 13 participants, the HT− group included 12 participants, and the RA group included 10 participants (3).

Participants underwent esophagogastroduodenoscopy (EGD), a procedure used to collect samples from the upper gastrointestinal tract, specifically the duodenum. Individuals who had taken antibiotics within one month prior to the procedure were excluded from the analysis to avoid confounding effects on the gut microbiome (3).

Within the HT+ group, six participants were receiving combined estrogen–progesterone therapy (five oral and one oral plus transdermal), while seven participants were receiving estrogen-only therapy (two oral, four transdermal, and one oral plus transdermal) (3).

2.5.1 *Results of the REIMAGINE study*

A statistically significant decrease in duodenal microbial diversity was observed in the HT− group compared with both the HT+ and reproductive-age (RA) groups, as measured by the Shannon diversity index—a commonly used metric that quantifies species richness and evenness within microbial communities such as the gut microbiome (3). The lower Shannon index values in HT− participants indicate a reduction in overall microbial diversity, including a loss of less abundant taxa within the duodenal microbial community (3).

In contrast, the HT+ group exhibited microbial diversity levels more similar to those observed in the RA group. This finding suggests that hormone therapy may help preserve gut microbial diversity following menopause, potentially reducing the likelihood of gut dysbiosis (3). Additionally, the core microbial composition of the duodenal microbiome was similar between HT+ and RA participants, whereas the microbial communities in HT− participants differed substantially (3).

Specifically, HT− participants exhibited lower abundances of bacteria belonging to the Bacteroidetes phylum, which includes the genus *Bacteroides*—a group previously discussed for its beneficial contributions to host metabolic and immune functions (3). At the same time, HT− participants showed increased levels of Proteobacteria, a bacterial group frequently associated with gut microbial imbalance and dysbiosis (3). Conversely, women in the HT+ group displayed a gut microbiome enriched with beneficial bacterial taxa such as *Prevotella*,

along with significantly lower levels of potentially harmful genera including *Escherichia* and *Klebsiella* (3).

Further evidence of microbial imbalance was observed using the Microbial Dysbiosis Index (MD-Index), a quantitative measure of dysbiosis. The HT− group exhibited a higher MD-Index value (0.5029) compared with the HT+ group (0.22), indicating greater microbial imbalance among participants not receiving hormone therapy (3).

Overall, the findings reported by Leite et al. (2022) suggest that hormone therapy may help preserve duodenal microbial diversity and reduce dysbiosis in postmenopausal women, potentially reflecting broader benefits for gut microbial health. However, these results should be interpreted cautiously. The REIMAGINE study included only 35 participants and was not specifically designed to evaluate AD outcomes or the effects of hormone therapy timing. As such, the findings should be considered preliminary and warrant further investigation in larger, targeted studies.

2.6 Alternative gut microbiome-targeted therapies for AD

As gut microbiota modulation has the potential to combat dysbiosis and mitigate AD pathogenesis or progression, multiple alternative gut microbiome-targeted therapies such as probiotics, synbiotics, and Fecal Microbiota Transplantation (FMT) have been developed (15).

2.6.1 *Prebiotics*

The potential therapeutic effects of prebiotics—such as omega-3–rich polyunsaturated fatty acids (PUFAs)—have been evaluated in numerous clinical studies for their ability to mitigate AD (15). One of the earliest investigations examining the role of such dietary interventions in AD patients was the OmegAD trial, conducted in Sweden in 2006 (15). This study was a randomized, double-blind, placebo-controlled clinical trial involving 204 participants (15).

The initial findings suggested that dietary supplementation with omega-3 fatty acids improved cognitive function in patients with very mild AD, although no significant benefit was observed in individuals with mild to moderate AD (15). Following the original trial, the OmegAD dataset was further analyzed through patient stratification to explore additional factors that might influence treatment outcomes. These secondary analyses examined variables including neuropsychiatric symptoms (n = 204), inflammatory markers (n = 35), plasma fatty acid profiles in relation to cognition and gender (n = 174), and indicators of oxidative stress and inflammation (n = 41) (15).

Beyond the OmegAD trial, interventional studies evaluating prebiotic or dietary supplementation strategies have been conducted in several countries, including Japan (n = 29), China (n = 46, 240, 240, and 63), the United States (n = 402, 39, and 29), Australia (n = 50), Malaysia (n = 36), Iran (n = 199), the United Kingdom (n = 76), and Germany (n = 49) (15). Many of these studies reported improvements in clinical outcomes among AD patients following prebiotic or omega-3 supplementation (15). However, some studies found no significant benefits in measures such as neuropsychiatric symptoms, inflammatory markers, cognitive performance, oxidative stress, or other related clinical indicators (15).

2.6.2 *Synbiotics*

Another microbiome-targeted therapeutic approach involves the use of synbiotics (15). Synbiotics are generally classified into two categories: complementary synbiotics and synergistic synbiotics (15). Complementary synbiotics consist of a combination of a prebiotic and a probiotic that are administered together but act independently to promote gut health. In contrast, synergistic synbiotics are designed so that the prebiotic component specifically supports the growth and metabolic activity of the paired probiotic strain, thereby enhancing its therapeutic effect (15).

Research on the use of synbiotics in transgenic AD animal models—genetically engineered organisms that carry genes associated with AD pathology—remains relatively limited (15). However, one study reported promising findings following the administration of a complementary synbiotic composed of inulin and a multispecies probiotic preparation containing *Bacillus natto*, *Bacillus coagulans*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Bifidobacterium breve* (15). In this study, synbiotic supplementation improved cognitive performance, reduced levels of amyloid-β42 (Aβ42), promoted neurogenesis, and decreased inflammatory responses in AD model organisms (15).

Interestingly, supplementation with inulin alone did not produce the same beneficial effects. This finding suggests that the combined action of prebiotics and probiotics within synbiotic formulations may be more effective than prebiotic supplementation alone in slowing the progression of Alzheimer's-related pathology (15).

2.6.3 *Fecal Microbiota Transplantation (FMT)*

Finally, fecal microbiota transplantation (FMT) has emerged as another gut microbiome–targeted intervention that may help slow the progression of AD (15). FMT involves transferring gut microbiota from a healthy donor to a recipient in order to restore microbial diversity and rebalance the gut microbiome. This approach may be particularly relevant for AD patients, who often exhibit reduced microbial diversity and gut dysbiosis.

Most studies investigating FMT in the context of AD have been conducted in animal models, particularly in germ-free (GF) mice or antibiotic-treated pseudo–germ-free mice (15). In a review of 19 FMT studies related to AD summarized by Zhang et al. (2023), several consistent outcomes were reported, including reductions in amyloid-β (Aβ) pathology, improvements in cognitive performance, restoration of gut microbiome composition, decreased neuroinflammation and gliosis, and, in some cases, reductions in tau pathology (15). Many of these studies used APP/PS transgenic mouse models, which carry genetic mutations associated with Alzheimer's pathology. FMT treatments varied in duration and administration method, though oral gavage—delivery of material directly into the stomach through a small tube inserted through the mouth—was commonly used (15). In several experiments, transplantation of microbiota from healthy or younger donor mice resulted in improvements in both neurological outcomes and behavioral performance in AD model animals (15).

However, several methodological limitations have been identified in some FMT studies. For example, in certain experiments the recipient animals' original gut microbiota were not fully

eliminated prior to transplantation, making it difficult to isolate the effects of the transplanted microbiota on disease outcomes (15). Such limitations highlight the need for more standardized experimental protocols.

Overall, although FMT and other gut microbiome–targeted therapies show promising potential, further research is needed to determine their long-term safety, efficacy, and optimal implementation. Continued investigation is also necessary to ensure that these interventions are carefully designed, rigorously tested, and reliably reported (15). Importantly, microbiome-based therapies may serve as complementary or alternative treatment strategies alongside hormone therapy. Such approaches could enable more personalized treatment options for AD, particularly for patients who may not be suitable candidates for hormone therapy.

2.7 Biological mechanisms underlying the Critical Window Hypothesis

The critical window hypothesis proposes that the timing of estrogen-based hormone therapy is a key factor influencing its effectiveness in reducing AD risk. In general, when hormone therapy is initiated well after the early postmenopausal period—typically defined as more than five years after menopause—it appears to provide little or no protective benefit against AD in postmenopausal women. Understanding why this timing is important requires examining both brain-centered and gut-centered biological mechanisms.

2.7.1 *Neurobiological mechanisms*

Current literature provides strong mechanistic evidence supporting brain-centered pathways following prolonged estrogen deprivation as a biological basis for the critical window hypothesis. During estrogen-based hormone therapy, administered estrogen binds to estrogen receptors and activates both genomic and non-genomic signaling pathways that support neuronal protection, regulate mitochondrial function, promote anti-inflammatory responses, and maintain synaptic integrity (22). As discussed earlier, when these protective mechanisms—such as anti-inflammatory signaling and synaptic maintenance—are disrupted, the brain becomes more vulnerable to neurodegenerative processes associated with AD.

In addition to their role in supporting glucose metabolism in the brain, estrogen receptors—including ERα, ERβ, and the membrane-associated receptor GPER1—are highly sensitive to the duration of estrogen deprivation (23). Following several years of menopause, prolonged estrogen deficiency can lead to the downregulation of these receptors, reducing both their abundance and functional responsiveness (23). Consequently, estrogen administered through hormone therapy becomes less able to activate receptor-mediated pathways that support neuroprotection. As a result, estrogen-based hormone therapy is generally less effective in reducing AD risk when initiated more than approximately five years after menopause. This receptor downregulation provides a plausible molecular explanation for the timing-dependent effects described by the critical window hypothesis.

Additional physiological changes may also contribute to the diminished neuroprotective effects of estrogen-based hormone therapy following prolonged estrogen deficiency. After extended periods without estrogen, neurons may

shift toward a ketogenic metabolic state, relying more heavily on fat-derived energy sources rather than glucose (23). This metabolic transition reflects reduced neuronal metabolic flexibility and may decrease neuronal responsiveness to estrogen signaling, thereby reducing the effectiveness of HRT.

There is also a cholinergic component to consider. Under normal conditions, estrogen helps maintain levels of acetylcholine, a neurotransmitter essential for memory formation and cognitive function (23). Prolonged estrogen deprivation can lead to reductions in acetylcholine signaling, which may further impair cognitive performance and limit the potential neuroprotective benefits of estrogen-based therapy (23). Together, these receptor, metabolic, and neurotransmitter-related changes provide a brain-centered explanation for why estrogen therapy often loses effectiveness when initiated beyond the critical window.

2.7.2 *Gut mechanisms*

Alternative hypotheses suggest that the gut microbiome and gut–brain axis may also influence estrogen responsiveness. Some researchers have proposed that, several years after menopause, estrogen may no longer significantly alter gut microbial composition, potentially limiting its downstream effects on the gut–brain axis and related neuroprotective pathways. However, current evidence provides limited support for this hypothesis.

For example, the previously discussed REIMAGINE study demonstrated that estrogen-based hormone therapy can modulate gut microbial diversity in postmenopausal women by reducing markers of gut dysbiosis (3). Although the study was not specifically designed to evaluate the effects of hormone therapy timing, its findings do not suggest a loss of gut microbiome responsiveness to estrogen in later postmenopausal stages.

Given the absence of clear evidence for a time-dependent decline in estrogen's gut-modulatory effects, current data remain insufficient to determine whether gut-based mechanisms meaningfully contribute to the critical window for reducing AD risk.

Evidence from a 2018 study by Kaliannan et al. further demonstrates that estrogen can influence gut microbiome composition and associated metabolic processes. In this study, administration of 17β-estradiol altered the gut microbiota in ways that reduced inflammatory and metabolic conditions linked to chronic disease in mice (24). Specifically, estrogen treatment modified the abundance of certain bacterial populations, which was associated with reduced susceptibility to inflammation and metabolic dysfunction (24).

Importantly, the study was conducted in ovariectomized mice—female mice whose ovaries had been surgically removed to eliminate endogenous estrogen production (24). Ovariectomy is commonly used as an experimental model to simulate the prolonged estrogen deficiency observed in women several years after menopause. This model allows researchers to investigate how estrogen therapy affects physiological systems, including the gut microbiome, following long-term hormone deprivation.

Taken together, the findings from both the REIMAGINE study and the Kaliannan et al.

(2018) study suggest that the gut microbiome may remain responsive to estrogen-based hormone therapy even after extended periods of estrogen deficiency. Table 1 compares the studies cited in this paper.

Table 1. Key studies examining hormone therapy, timing of initiation, and AD risk

Study	Study Type	Population	Hormone Therapy Type	Timing Relative to Menopause	Main Findings
Song et al. (2025)	Systematic review & meta-analysis	~7 million women	Estrogen-only vs combined therapy	Early initiation (3–5 years postmenopause)	Estrogen-only therapy associated with reduced AD risk; combined therapy associated with increased risk
Nerattini et al. (2023)	Systematic review & meta-analysis	RCTs (n=42,062) + observational cohorts	Estrogen-only vs combined therapy	Midlife initiation vs ≥65 years	Estrogen-only therapy associated with ~32% reduced dementia risk; combined therapy increased dementia risk in older women
Leite et al. (2022)	Observational microbiome study	35 women (HT+, HT−, reproductive age)	Estrogen ± progesterone	Postmenopause	Hormone therapy associated with higher gut microbial diversity and reduced dysbiosis
Kaliannan et al. (2018)	Animal model study	Ovariectomized mice	17β-estradiol	Simulated postmenopausal estrogen deficiency	Estrogen altered gut microbiome composition and reduced inflammatory/metabolic disease markers

2.8 Gut-related modifiers of HRT efficacy

This review does not suggest that long-term gut deterioration has no influence on the effectiveness of hormone therapy after the critical window period. Prolonged estrogen deficiency may still create intestinal conditions that alter the response to hormone therapy. Therefore, it is important to consider factors such as chronic inflammation and disruptions in enterohepatic estrogen circulation as potential secondary contributors to changes in hormone therapy effectiveness.

2.8.1 *Inflammation*

As discussed earlier, prolonged estrogen deficiency can compromise the integrity of the intestinal barrier, allowing harmful substances from the gut to enter the bloodstream. This process can trigger systemic inflammation and may contribute to pathways associated with AD risk. After years of estrogen deficiency, it is plausible that some individuals may develop a gut–brain axis characterized by persistent inflammatory signaling, which could potentially reduce the responsiveness to estrogen-based hormone therapy.

However, this hypothesis remains speculative, as there is currently limited research directly examining whether gut–brain axis dysfunction becomes irreversible in women who initiate hormone therapy later after menopause. Consequently, while inflammation may contribute to reduced therapeutic benefits when hormone therapy is initiated beyond the critical window, existing evidence suggests it is more likely a secondary contributing factor rather than a primary cause of the diminished effectiveness of late hormone therapy.

2.8.2 *Enterohepatic circulation*

Sustained gut dysbiosis may also impair the gut microbiota's ability to regulate enterohepatic estrogen circulation and recycling, providing

another possible explanation for why estrogen's neuroprotective effects may become limited over time. Although such disruptions could alter estrogen bioavailability, pharmacological doses of estrogen administered through hormone therapy may partially bypass these limitations. Therefore, impaired enterohepatic estrogen circulation is more likely to act as a modifying factor rather than a primary cause of reduced hormone therapy effectiveness.

If disruptions in estrogen circulation had a dominant effect on treatment outcomes, it would be reasonable to expect that postmenopausal women with gut dysbiosis would fail to respond to estrogen therapy for AD prevention regardless of when treatment was initiated. However, several studies have demonstrated that estrogen therapy can still improve gut microbial balance and reduce AD risk under certain conditions. These findings suggest that although dysbiosis may influence estrogen bioavailability, it is unlikely to completely eliminate hormone responsiveness during the critical window.

Taken together, the most strongly supported explanation for the reduced effectiveness of hormone therapy beyond the five-year critical window involves neuronal estrogen receptor downregulation and estrogen-dependent metabolic changes in the brain. Gut-related factors—including dysbiosis, inflammation, and disruptions in enterohepatic estrogen circulation—may influence treatment outcomes to a lesser extent, but current evidence suggests they function primarily as secondary contributors rather than the principal drivers of diminished hormone therapy efficacy when treatment is initiated later after menopause.

3. Discussion

A decline in estrogen levels following menopause increases women's risk of cognitive decline and the development of neurodegenerative diseases, particularly AD. Current literature suggests that HRT may reduce AD risk under specific conditions. Evidence indicates that estrogen-only hormone therapy—especially when administered orally for ~ three to five years and initiated within five years of menopause—is associated with a lower risk of AD. In contrast, progesterone-only or combined estrogen–progesterone therapies, as well as hormone therapy initiated more than five years after menopause, appear to provide little benefit and in some cases may be associated with an increased risk of AD.

Research also suggests that hormone therapy may influence gut microbiome composition, increasing microbial diversity and reducing the likelihood of gut dysbiosis. Microbial imbalance in the gut has been observed in individuals with AD and has been linked to pathways associated with neuroinflammation and neurodegeneration.

Although research remains limited regarding the most effective hormone therapy formulation, delivery method, and timing for optimizing gut microbiome health, existing evidence consistently indicates that early initiation of estrogen-only therapy during the early postmenopausal period provides the most reliable reduction in AD risk. This timing-dependent neuroprotective effect is largely explained by brain-centered mechanisms, including estrogen receptor signaling and metabolic regulation within neurons. Prolonged estrogen deprivation can lead to receptor downregulation and metabolic changes that reduce the brain's responsiveness to hormone

therapy initiated later in life.

In addition to hormone therapy, microbiome-targeted interventions—such as probiotics, synbiotics, and fecal microbiota transplantation—have shown promise in restoring microbial balance and addressing gut dysbiosis. These approaches may serve as complementary strategies for improving gut health and potentially reducing AD risk, particularly in individuals who are unable to undergo hormone therapy.

3.1 Gaps in existing research

Although numerous studies have examined the effects of hormone replacement therapy (HRT) on cognitive decline after menopause, as well as its potential influence on the gut microbiome and gut dysbiosis, relatively few studies have investigated the relationship between these two areas. In particular, there is a lack of research directly examining whether hormone therapy can restore gut microbiome balance in postmenopausal women.

Existing studies also provide limited analysis of how different hormone therapy formulations (estrogen-only versus combined estrogen–progesterone therapy), delivery methods (oral versus transdermal), or timing of initiation (early versus late after menopause) may influence the restoration of gut microbial balance. Notably, studies specifically evaluating the impact of early versus late initiation of hormone therapy on gut microbiome health remain scarce.

Furthermore, current research has not yet clearly established whether gut dysbiosis and reduced microbial diversity observed in individuals with AD are driven directly by the disease itself or instead reflect the combined effects of aging, comorbid conditions, or medications commonly used in older populations. Addressing these gaps will be important for clarifying the role of hormone therapy and the gut microbiome in AD risk and progression.

Beyond these conceptual gaps, there are also quantitative gaps in this field. Few studies have collected data characterizing the decline of estrogen receptors (ERα/ERβ) in the human hippocampus during the course of menopause and no clear thresholds have been identified establishing the degree to which hormone therapy efficacy diminishes as estrogen receptor density and circulating estrogen levels decline. Similarly, the interactions among hormone therapy dosage, timing of initiation, neuroinflammation burden, and gut microbial diversity have not yet been quantified in terms of measurable relationships in existing research.

In terms of methodology, long-term clinical trials are scarce, as chronic conditions such as AD develop over the course of many years, and treatments such as hormone therapy are still fairly recent. Moreover, significant heterogeneity across studies — including differences in age stratification beyond simple early/late timing, APOE ε4 genetic risk status, diet, physical activity, medication use, and baseline microbiome composition — introduces uncontrolled, confounding variables that may influence both cognitive and gut outcomes, complicating the feasibility and interpretation of long-term hormone therapy trials. Numerous existing studies also rely upon cross-sectional or retrospective designs rather than long-term follow-up, which further restricts the ability to track longitudinal changes in patient hormone levels, cognition, and gut microbiome

composition. Many studies did not incorporate interaction testing (ex: therapy type × timing), longitudinal mixed-effects modeling, or correction for multiple hypothesis testing, increasing the risk of false-positive subgroup findings and limiting confidence in reported associations. Current conclusions also depend disproportionately on animal models —which may not directly translate to human physiology. Additionally, few investigations simultaneously assess the gut and brain within the same participants, limiting integrated understanding of gut-brain interactions.

Importantly, current findings linking hormonal therapy to gut microbiome changes remain exploratory rather than confirmatory as human microbiome findings rely primarily on small cohorts, seen most notably in the REIMAGINE study conducted at Cedars-Sinai Medical Center where the cohort size was 35 participants. These experimental designs are insufficient when establishing causality in broader human populations. Therefore, microbiome-related conclusions in this review should be interpreted as hypothesis-generating rather than definitive.

Together, these conceptual, quantitative, and methodological limitations in existing literature place a certain constraint on the strength of this paper's findings. Furthermore, the relative strength of evidence supporting brain-centered versus gut-centered mechanisms is comparable in that both rely largely on indirect or preliminary data. Hence, currently neither pathway can be defined as causal and interpretations of relative mechanistic importance should be taken as provisional.

3.2 Proposed experimental test of the Critical Window Hypothesis

Because current evidence cannot clearly determine whether the earliest loss of estrogen responsiveness occurs primarily in neuronal signaling pathways or in gut microbiome–mediated pathways, a study capable of simultaneously measuring both systems is required. An experimental design that stratifies participants by time since menopause and directly compares brain and gut responses to estrogen therapy could provide a decisive test of these competing hypotheses. Although the proposed study compares gut and brain responses to estrogen therapy, these systems are not biologically independent. The gut microbiome and the brain interact through the gut–brain axis, and microbial metabolites, immune signaling, and hormonal metabolism can influence neural function. Therefore, the goal of this experimental design is not to treat gut and brain mechanisms as mutually exclusive, but rather to determine which system shows the earliest or strongest loss of estrogen responsiveness following prolonged estrogen deprivation.

To move beyond treating the critical window hypothesis as a broad epidemiologic observation, future research should conceptualize it as a timing-dependent problem of tissue-specific estrogen responsiveness that can be experimentally tested. A directly implementable approach would involve a timing-stratified, randomized estrogen intervention trial in postmenopausal women designed to determine whether the critical window arises primarily from brain-dependent mechanisms, gut-dependent mechanisms, or a combination of both.

Participants would be recruited and stratified based on the time elapsed since menopause:

early (<3 years), mid (5–8 years), and late (>10 years). These categories represent three biological states reflecting progressively longer durations of estrogen deprivation. To minimize genetic confounding, individuals carrying the APOE ε4 allele—a major genetic risk factor for Alzheimer's disease associated with altered lipid metabolism, neuroinflammation, and amyloid accumulation—would be excluded from the initial trial. Because APOE ε4 status may independently influence both neurodegeneration and estrogen signaling pathways, its inclusion could obscure the specific relationship between estrogen responsiveness and the timing of hormone therapy initiation. Restricting the initial study population to non-carriers would allow researchers to more clearly isolate the physiological mechanisms underlying the critical window hypothesis. Subsequent studies could then examine whether similar patterns occur in APOE ε4 carriers.

Within each timing group, participants would then be randomly assigned to receive either oral estrogen-only hormone therapy or a placebo. Randomization is essential because it ensures that any observed differences in outcomes can be attributed to the hormone therapy rather than lifestyle, medical history, or other confounding factors.

The central component of this experiment would involve longitudinal, simultaneous measurement of both brain and gut responses to estrogen therapy over an extended period of time. In the brain, researchers would measure indicators of neuronal function and neurodegeneration, including brain glucose metabolism using FDG-PET imaging, neuroinflammatory biomarkers, structural and functional MRI outcomes, and cognitive performance across domains such as memory, learning, and executive function. In the gut, investigators would examine microbiome diversity and composition through stool metagenomic sequencing, microbial estrogen metabolism pathways, gut permeability biomarkers ("leaky gut" indicators), and systemic inflammatory cytokine levels.

By comparing how these systems respond across the three menopause timing groups, researchers could determine whether estrogen responsiveness is lost simultaneously across tissues or whether the decline occurs in a tissue-specific manner. If both gut and brain biomarkers improve only in the early postmenopausal group but not in the mid or late groups, this would suggest a global loss of estrogen responsiveness throughout the body after prolonged estrogen deprivation. In contrast, if gut microbiome measures improve in late postmenopausal women while brain biomarkers do not, this would indicate that the gut remains estrogen-responsive longer than the brain and that the critical window is primarily driven by brain-specific mechanisms. Conversely, if gut responses decline earlier than brain responses, this would suggest that gut-mediated pathways contribute significantly to the timing-dependent loss of hormone therapy effectiveness.

Such a study would allow researchers to directly test whether the critical window hypothesis is primarily governed by brain-centered neurobiological mechanisms or by gut–brain axis mechanisms, thereby moving the concept from observational association to experimentally validated physiology.

4. Conclusion

The decline of estrogen after menopause represents a critical biological turning point that may increase women's vulnerability to AD. Evidence reviewed here suggests that estrogen-based hormone therapy—particularly oral estrogen initiated early after menopause—may reduce Alzheimer's risk while also improving gut microbial diversity and limiting dysbiosis. However, the precise mechanisms underlying this protective effect remain unresolved. Although brain-centered pathways currently provide the strongest explanation for the critical window hypothesis, emerging data indicate that the gut microbiome and gut–brain axis may also play an important modulatory role.

A central gap in the literature is that brain mechanisms and gut mechanisms have largely been studied in isolation. This review highlights the importance of integrating these fields by examining how estrogen therapy influences both neurobiological processes and microbial ecosystem dynamics. Addressing this gap requires moving beyond observational evidence toward direct experimental testing of tissue-specific estrogen responsiveness.

The proposed timing-stratified randomized estrogen intervention trial provides a practical framework for doing so. By simultaneously measuring brain biomarkers of neurodegeneration and gut microbiome responses across early, mid, and late postmenopausal stages—while excluding APOE ε4 carriers to control for genetic risk—such a study could directly determine whether the critical window arises primarily from loss of estrogen responsiveness in the brain, the gut, or both. Establishing this mechanism would transform the critical window hypothesis from a descriptive clinical observation into a testable physiological model.

The distinction between gut-mediated and brain-mediated mechanisms underlying the critical window hypothesis has important therapeutic implications. If loss of hormone therapy responsiveness in late postmenopausal women is driven primarily by gut microbiome dysbiosis, the problem may be reversible through microbiome-targeted interventions such as probiotics, synbiotics, dietary modulation, or fecal microbiota transplantation. These approaches could potentially restore microbial balance and re-establish estrogen responsiveness even after prolonged estrogen deprivation. In contrast, if the critical window arises from brain-specific mechanisms such as neuronal estrogen receptor downregulation or metabolic reprogramming, microbiome interventions alone would be insufficient. In this case, therapeutic strategies would need to focus on bypassing or compensating for lost estrogen signaling through approaches such as selective estrogen receptor modulators, brain-penetrant estrogen analogs, or metabolic support therapies. Determining which of these mechanisms predominates is therefore not only a biological question but also a critical step in identifying actionable treatment strategies for reducing AD risk in postmenopausal women.

Ultimately, advancing Alzheimer's prevention in women will require precisely defining when estrogen therapy works, why its benefits decline with time, and how gut–brain interactions influence this process. Resolving these questions will not only clarify the biology of the critical window but may also open the door to combined hormone and microbiome-based interventions capable of modifying AD risk

before irreversible neurodegeneration occurs.

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